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SUBJECT: COMMENTS ON THE LISTING OF ATRAZINE ON THE ICCVAM EDWG PROPOSED LIST OF SUBSTANCES FOR VALIDATION OF *IN VITRO* ENDOCRINE DISRUPTOR METHODS.

Atrazine was selected as one of 9 pesticides on the ICCVAM EDWG proposed substance for validation of ER and AR binding and transcriptional activation assays. In that regard, atrazine is listed in Appendix A (ICCVAM EDWG Proposed Substance for Validation of ER and AR Binding and Transcriptional Activation Assays) as a chemical with an anticipated in vitro response in the ERTA and ARTA and/or binding as positive. The basis for these conclusions can be purported found in a summary file of *in vitro* data for NICEATM (http://iccvam.niehs.nih.gov/methods/endodocs/ed_brd.htm). However, when one examines the basis for these assumptions, the weight of evidence would support that atrazine does not bind to the estrogen receptor either in ERTA or ER cytosol. In fact, atrazine did not bind to the human ER_ transfected to CHO-K1 cell (Otsuka Pharmaceutical, 2001), human ER transfected to HeLa cell (Balaguer et al., 1996), human ER_ transfected to MCF-7 cells (Connor et al., 1996; Soto et al., 1995), and human ER transfected to yeast (Graumann et al., 1999). The only positive response was observed in rat ER transfected to yeast (Petit et al., 1997). Besides, the work by Graumann et al. (1999) with human ER transfected with yeast, Connor et al. (1996) also used an estrogen-dependent recombinant yeast strain PL3; these authors found estrogen-dependent PL3 yeast strain was not capable of growth on minimal media supplemented with atrazine in place of E2. Therefore, it would appear more appropriate to list atrazine as negative in the ERTA and /or binding assays and unknown in the ARTA and /or binding assays. In addition, atrazine under in vitro data (NICEATM) in Appendix A, binding; atrazine is identified as weakly ER+/AR+; there not basis for this supposition as atrazine was found not to bind to ER isolated from rat uterus (Tennant et al., 1994).

Also in Appendix A, under studies proposed by the U.S. EPA, atrazine was slotted for an AR binding assay, pubertal male assay and potentially for the *in utero* through lactation assay. The AR binding assay, although anticipated to be negative, may add value if completed, the pubertal male has been completed (Stoker et al., 1999), and the *in utero* through lactation assay as a screen is far from being validated, is not needed as a test, and should not be used for evaluating the substance on the ICCVAM EDWG proposed substances list.

Thank you for your consideration of these comments.

Sincerely Yours,

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